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Signaling events mediated by a3b1 integrin are essential for mammary tumorigenesis

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ORIGINAL ARTICLE

Signaling events mediated by α3β1 integrin are essential for mammary tumorigenesis

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The constitutive activation of β-catenin signaling in the mammary basal epithelial cell layer in transgenic K5ΔNβcat mice leads to basal-type tumor development. Integrins of the β1 family and integrin-mediated signaling events have an important role in breast tumor growth and progression. We show here that the deletion of α3β1 integrin, a major laminin receptor, from the basal layer of the mammary epithelium of K5ΔNβcat mice completely prevented the tumorigenesis induced by β-catenin signaling. Moreover, the depletion of α3β1 integrin from a spontaneously transformed mouse mammary basal epithelial cell line (MEC) prevented the cells from forming colonies in soft agar and greatly reduced tumor development in orthotopic grafts. Inhibition of the integrin signaling intermediates Rac1 or PAK1 (P21-activated Kinase 1) in MEC affected tumor cell growth in soft agar, whereas the expression of activated forms of these effectors in α3-depleted cells rescued the capacity of these cells to grow in non-adherent conditions. Similarly, the tumorigenic potential of α3-depleted cells was restored by the expression of activated PAK1, as assessed by orthotopic transplantation assay. In three-dimensional Matrigel culture, MEC survival and proliferation were affected by the depletion of α3β1 integrin, which also significantly decreased the activation of focal adhesion kinase (FAK), mitogen-activated protein kinase (MAPK) and c-Jun NH2-terminal kinase (JNK). Our data suggest that the activation of signaling cascades downstream from α3β1 and involving the Rac1/PAK1 pathway, MAPK and JNK, promotes prosurvival and proproliferative signals required for the malignant growth of basal mammary epithelial cells, providing further insight into the molecular mechanisms underlying breast cancer initiation and progression.

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Keywords: integrin signaling; basal-like breast cancer; mouse models of breast cancer; Wnt/β-catenin pathway; survival; proliferation

INTRODUCTION

Breast cancers display high levels of cellular and molecular heterogeneity, which influences their responsiveness to treatment.[1] Breast tumors have been classified into four major molecular subtypes on the basis of their expression profiles, and each of these subtypes may be considered as a separate disease.[2–5] One of these subtypes, basal-like mammary carcinoma, accounts for about 15–20% of breast cancers and is

terminal kinase (JNK) and mitogen-activated protein kinase (MAPK).[9,10] Moreover, integrins have been reported to be involved in various types of cross-talk with growth factor receptor-mediated signaling.[11]

Integrin and associated intracellular signaling effector expression levels and/or activity are often modified during tumor development, suggesting that the adhesion machinery has a role in malignant transformation and tumor progression.[10,12–14]

About the Journal

On this page: Aims & Scope | Impact Factor | Abstracted/indexed in | ISSN and E-ISSN | Newsfeeds

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Cell Biology: Arnoud Sonnenberg

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Cell-matrix adhesion

We have been studying the a3b1 and a6b4 integrins and how they mediate the physical attachment of cells to laminin, which is a component of the extracellular matrix. Although the two molecules also associate with the tetraspanin CD151 at the cell surface, it is in particular a3b1 that binds directly and robustly to this protein and it is associated with the actin cytoskeleton in focal adhesions. The a3b1/CD151 ternary complex is also present in the glomerulus of the kidney. We previously generated genetically modified mice lacking a3b1 or CD151 in the glomerulus, and found that they develop progressive renal failure with a decreased glomerular filtration capacity. We have shown that blood pressure is an important determinant of this progressive disease and are now working on deciphering the underlying molecular mechanisms involved. We are also studying the role of a3b1/CD151 in skin cancer and have identified an important function of this complex in the development and progression of skin tumors by controlling the migration of

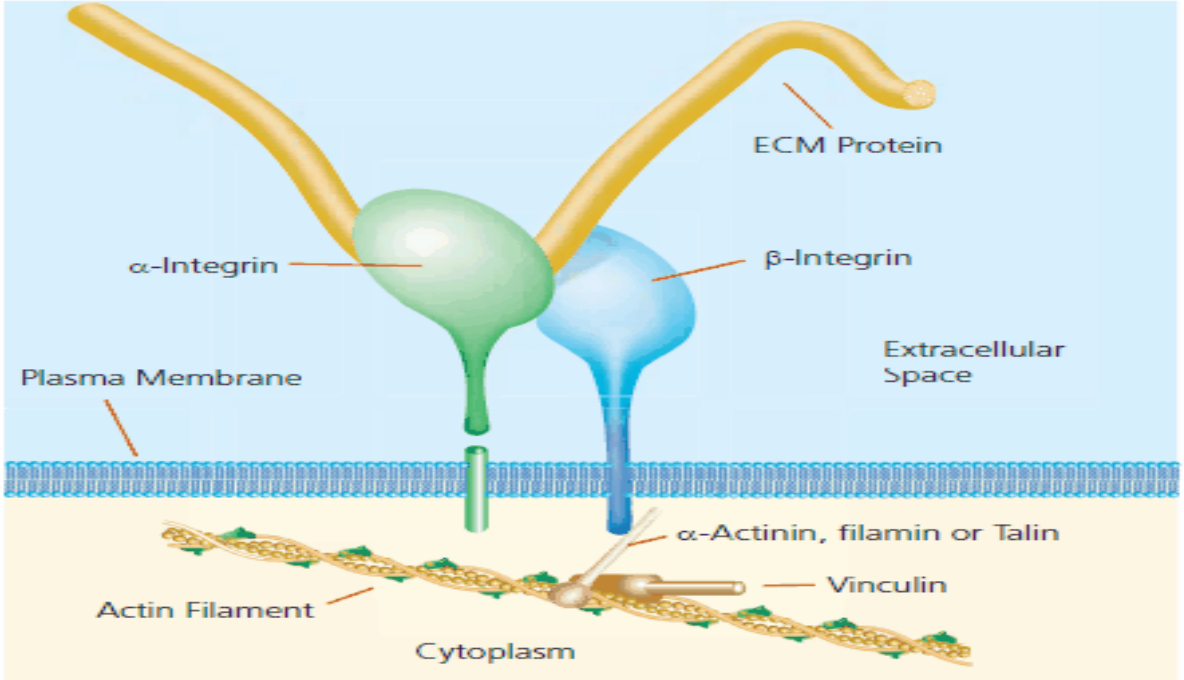
and clinical expertise, development and training.

❖ integrins definition

- Integrins are transmembrane receptors formed by non-covalently bound α and β subunits and connect the extracellular matrix to the cytoskeleton proteins include fibronectin, collagen and laminin

- It has been identified as many as 18 α and 8 β subunits, from which 24 different functional integrins in human

- $\alpha 3\beta 1$ ⟶ Laminin-5 (Laminin-332)



❖ in vivo and in vitro study

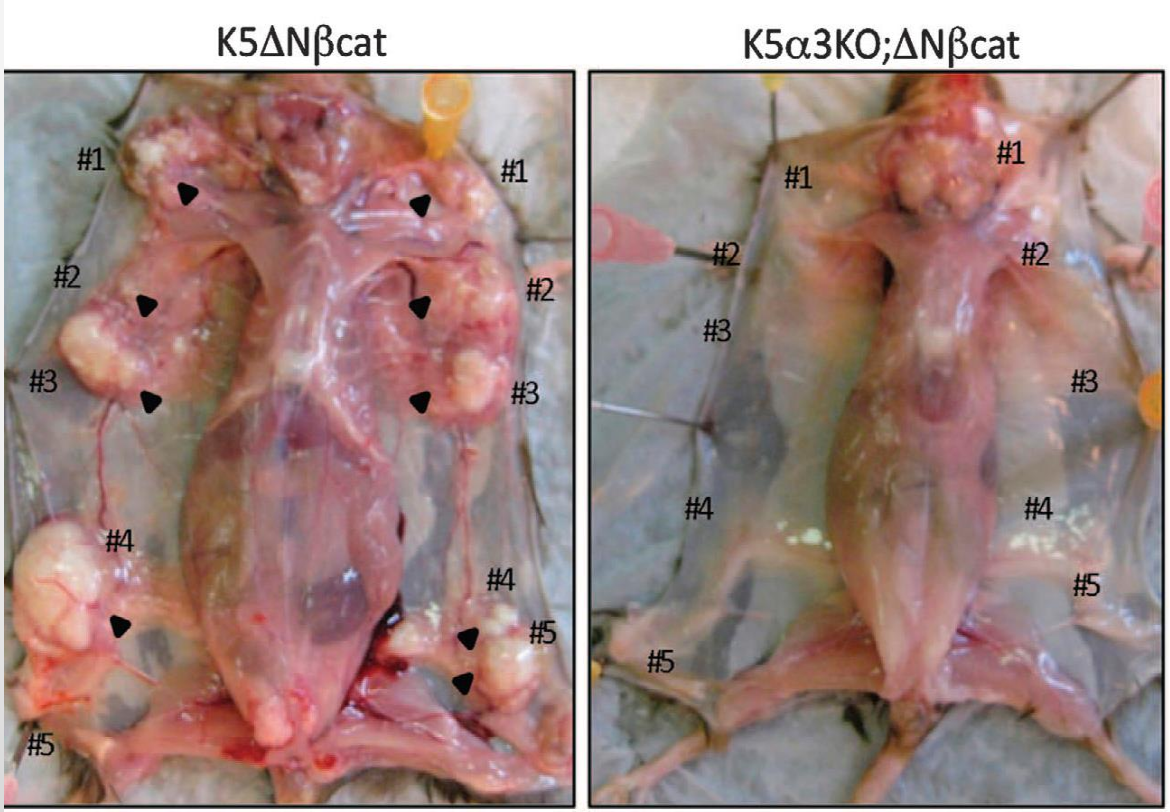
- ❑ K5ΔN57 βcat transgenic mice → N-terminally truncated (lacking amino acids 1 to 57) mouse β-catenin
  - ➢ Mouse mammary basal epithelial cell line (MEC)

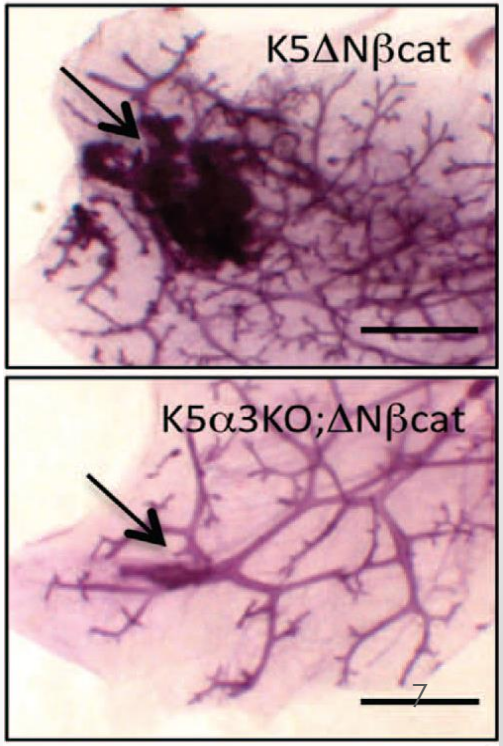
- ❑ K5α3KO; Δ Nβcat knockout mice → The depletion of integrin α3from the mammary basal cell layer of K5ΔNβcat mice
  - ➢ Mouse mammary basal epithelial cells lacking integrin α3 (α3KO-MEC)

❖ Photographs & carmine alum staining of mammary glands

- ✓ The depletion of integrin α3β1 from the mammary basal cell layer of K5DNβcat mice (K5α3KO;DNβcat mouse mutants) completely prevented tumorigenesis

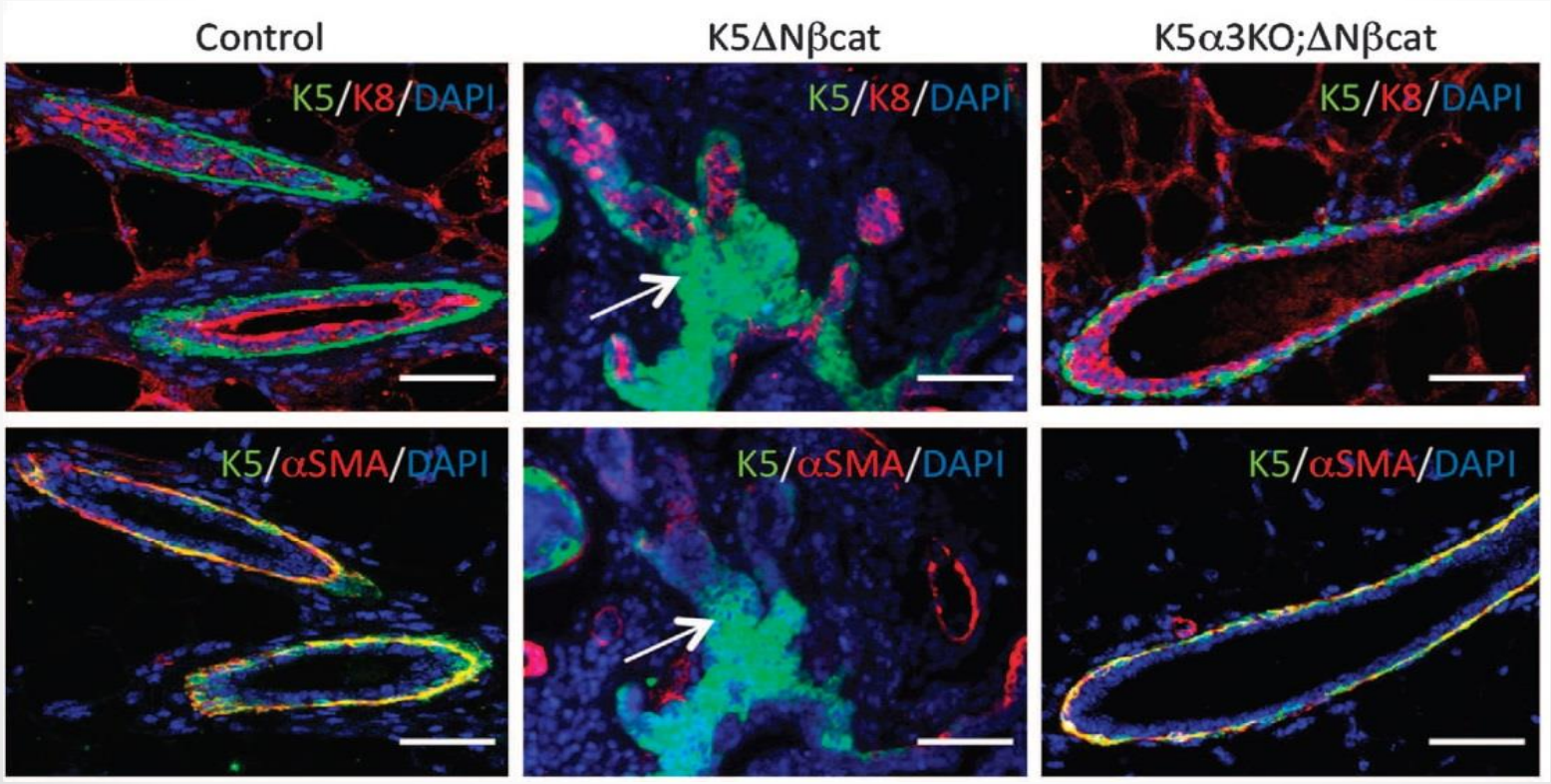
- ✓ The luciferase reporter assay is commonly used as a tool to study gene expression at the transcriptional level. Luciferase is an oxidative enzymes that produce bioluminescence, and is usually distinguished from a photoprotein.





❖ Double immunofluorescence labeling of sections through mouse mammary glands

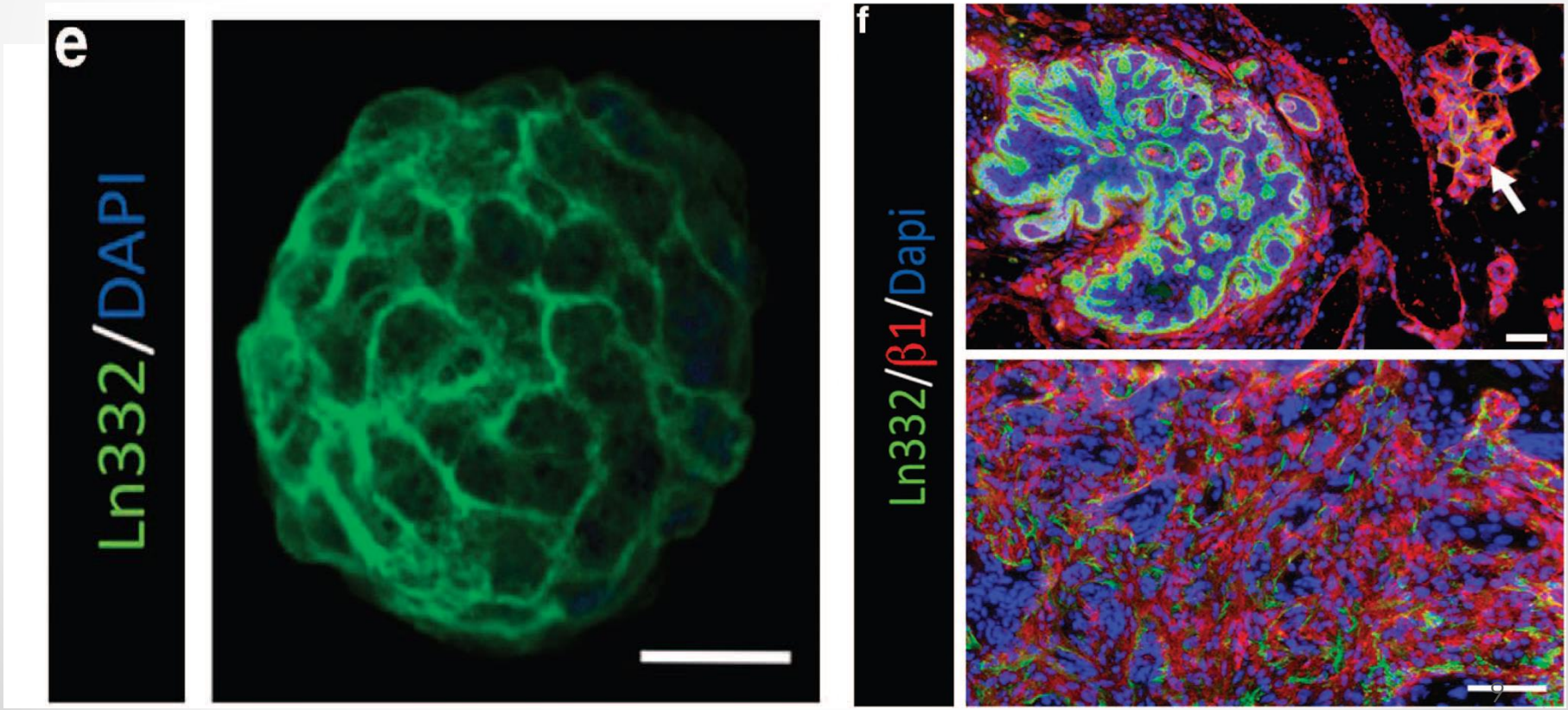
- ✓ antibodies against keratin 5 (K5) and keratin 8 (K8) & antibodies against K5 and α-smooth muscle actin (αSMA)
- ✓ The difference in β-catenin activation between α3KO-MEC and MEC was not statistically significant, suggesting that integrin α3β1deletion did not prevent β-catenin-Tcf-driven transcription.



- investigated the capacity of MEC and α3KOMEC cells to grow in non-adherent conditions, in soft agar.

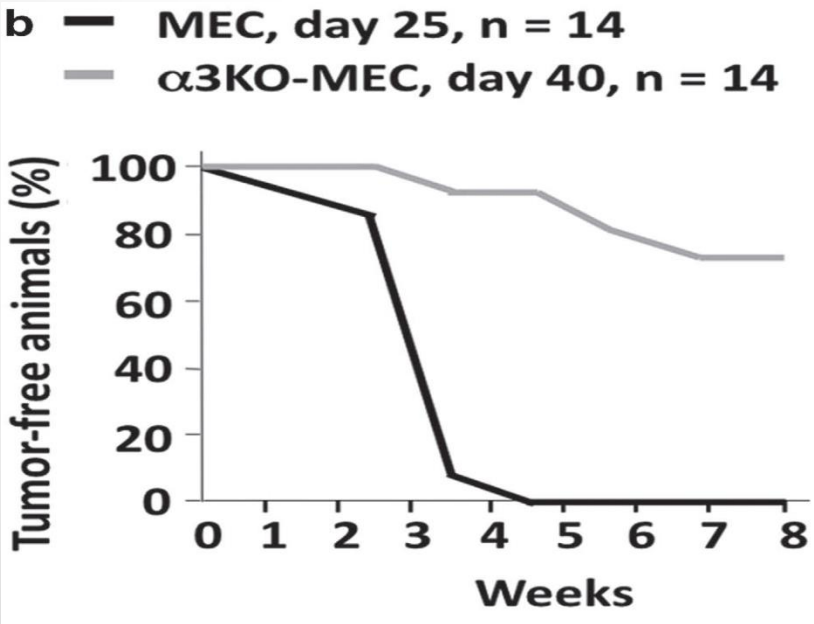
  - ✓ Anchorage-independent proliferation is a hallmark of cancer cells

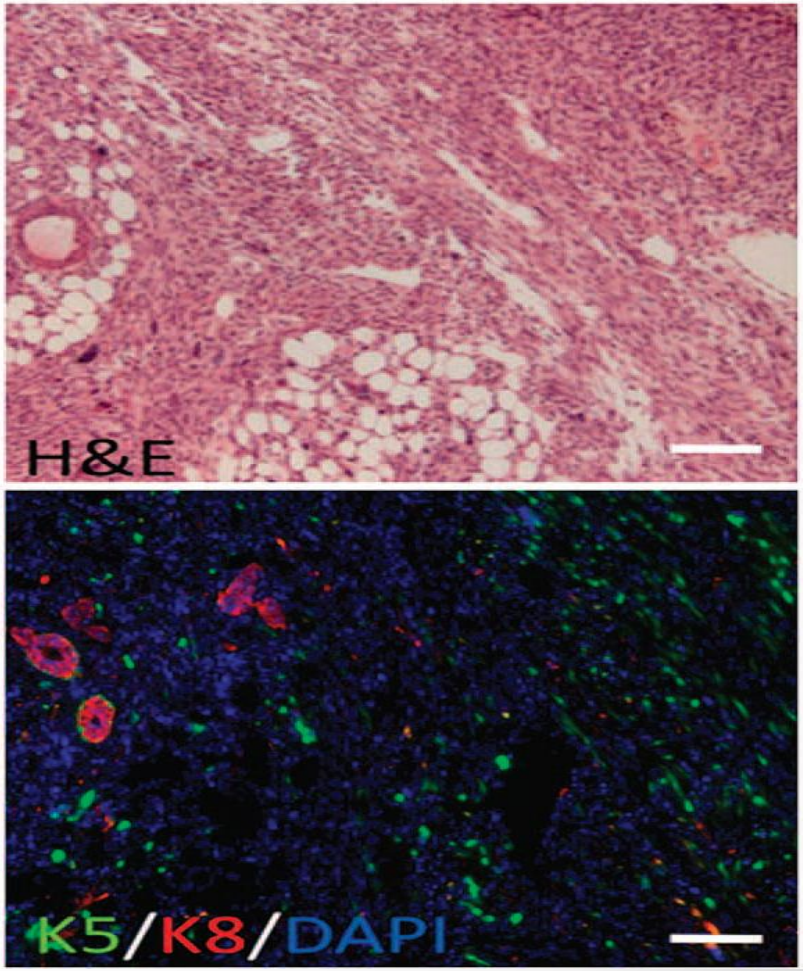
  - ✓ ability of these cells to synthesize and secrete Ln332 suggesting a possible role for Ln332 in tumorigenesis



❖ Hematoxylin and eosin (H&E) staining, immunofluorescence labeling with antibodies against K5 and K8

The injection of MEC cells into the fat pads led to the development of tumors consisting essentially of spindle-shaped K5-positive

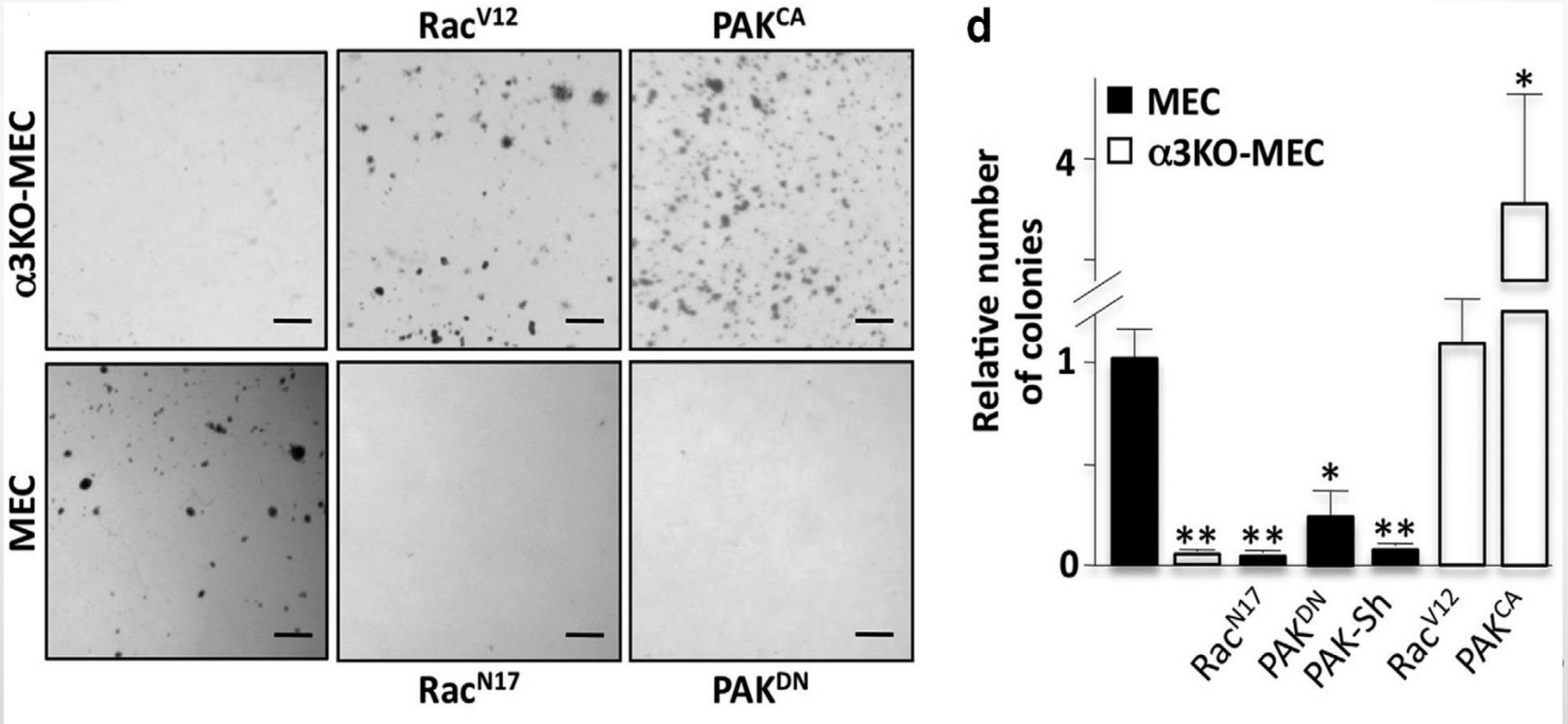




❖ α3β1 -Mediated signaling is required for tumor development from MEC in soft agar

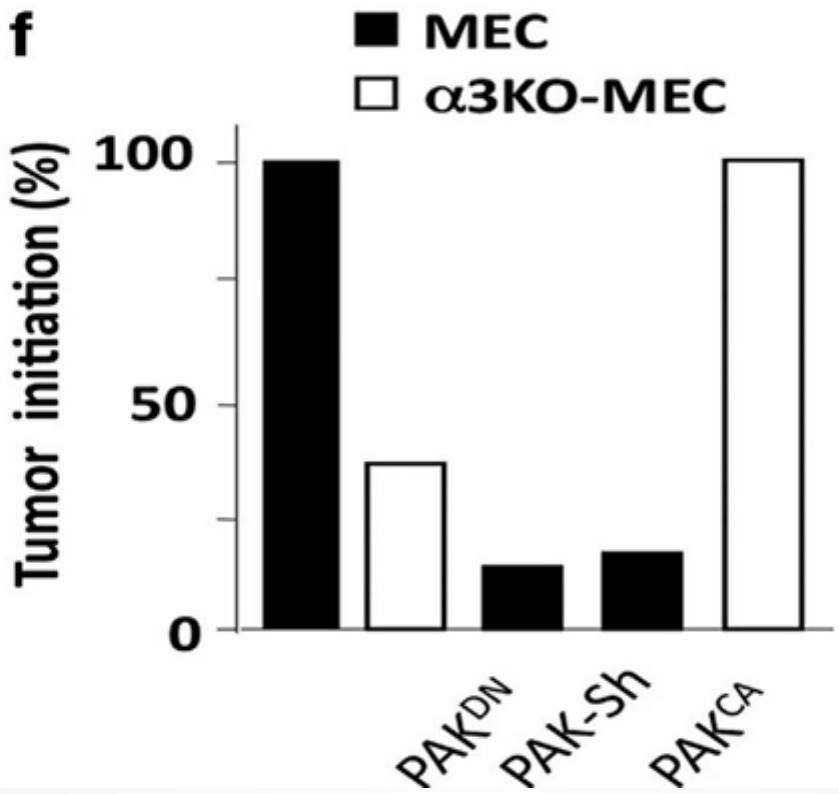
- ✓ We have shown that the focal adhesion kinase (FAK)& Rac or its effector p21-activated kinase (PAK) pathway, FAK/Rac1/PAK1 pathway, is activated downstream from integrin α3β1

- ✓ The expression of a constitutively active form of Rac1 (RacV12) or of a catalytically active form of its effector, PAK1 (PAKCA), restored the capacity of a3KO-MEC cells to form colonies in soft agar
- ✓ The treatment of MEC cells with the Rac inhibitor NSC23766, the expression of a dominant-negative form of Rac1 (RacN17) or PAK1 (PAKDN), or PAK1 silencing by shRNA (PAK-sh) in MEC cells led to an inhibition of colony formation in soft agar



❖ Integrin α3β11-mediated stimulation of the Rac1/PAK1 pathway promotes tumorigenic potential

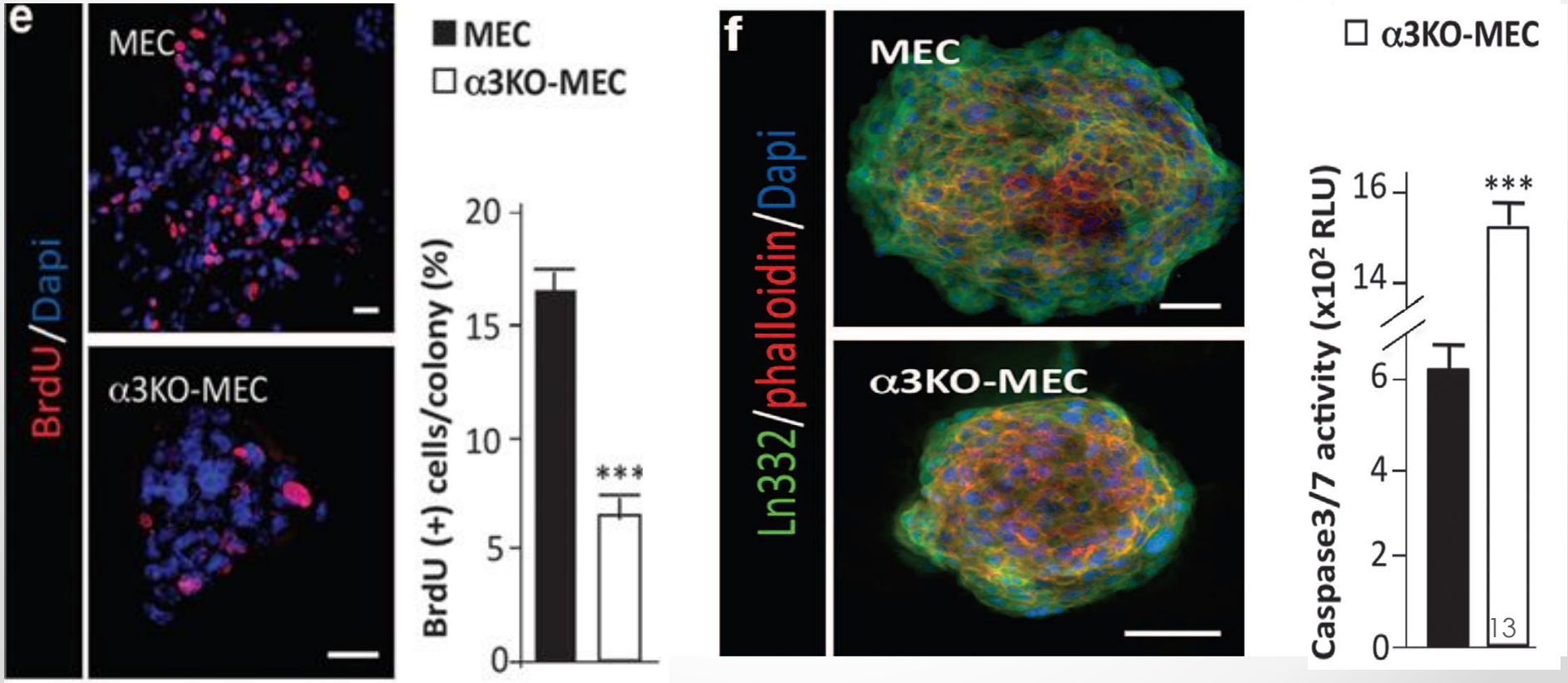
- ✓ The deletion of itga3 from the mammary basal cell layer in K5DNbcat mice halved the level of FAK phosphorylation
- ✓ the inhibition of PAK1 activity in MEC cells greatly decreased the formation of tumors from cells grafted into the mammary fat pad, whereas the expression of PAKCA in a3KO-MEC cells restored their tumorigenic potential



❖ k5α3KO-MEC have a low colony-forming capacity in 3D Matrigel

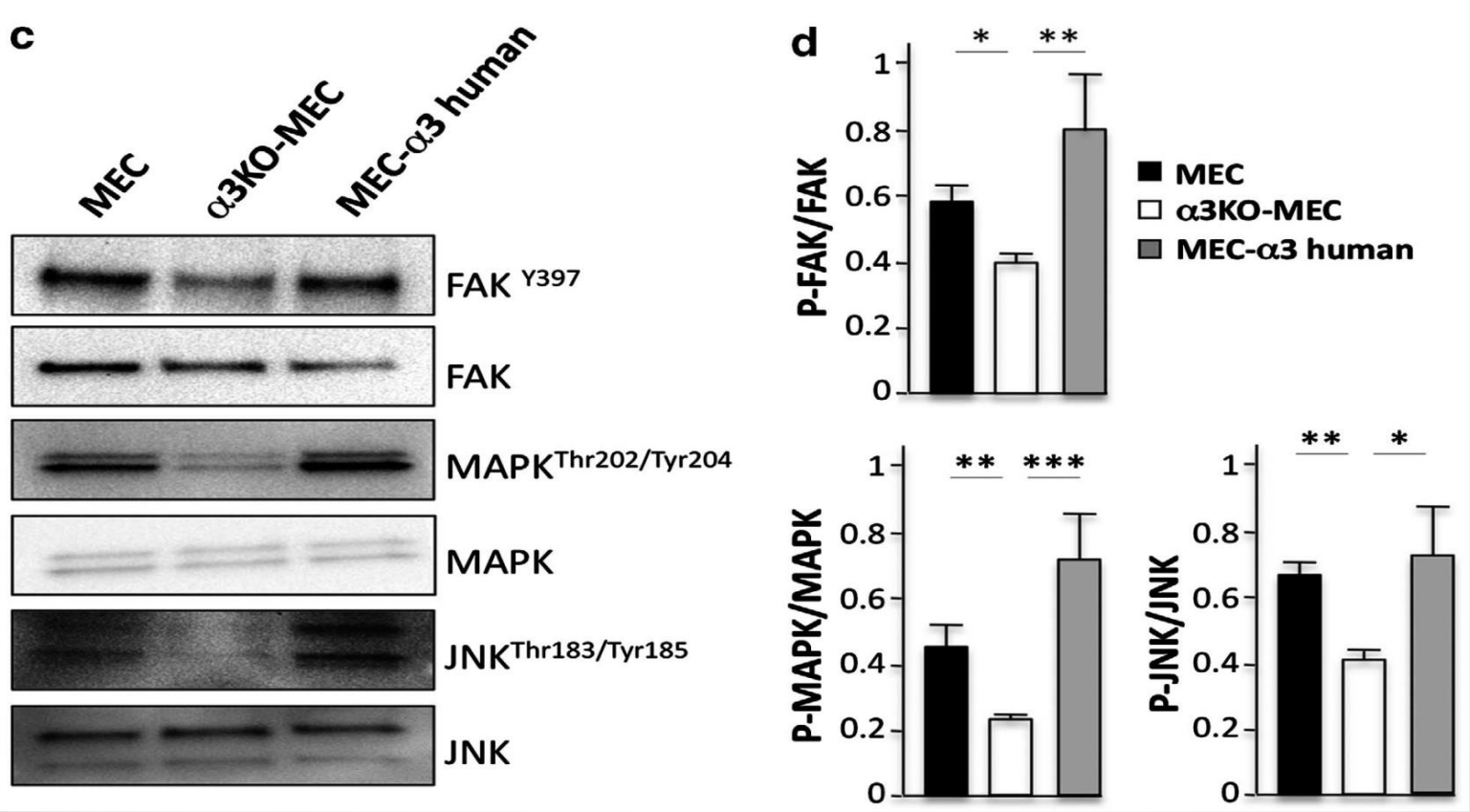
- ✓ The treatment of MEC cells with the Rac inhibitor NSC23766 or the expression of a dominant-negative form of Rac1 (RacN17) in MEC cells led to an inhibition of colony growth, Furthermore, the expression of a constitutive active form of Rac1 (RacV12) in a3KO-MEC cells restored the capacity of these cells to form colonies

- ✓ the lower capacity of k5α3KO-MEC cells to form colonies was associated with higher levels of caspase-3/7 activity at early stages of culture and lower proliferation rates



❖ The loss of integrin α3β1 results in the impaired activation of FAK, MAPK and JNK

- ✓ absence of integrin a3b1 resulted in much lower levels of MAPK and JNK phosphorylation



❖ DISCUSSION

- ✓ deletion of the itga3 gene from the mammary epithelial basal layer of K5DNbcat mice prevented the mammary tumorigenesis induced by the activation of b-catenin signaling
- ✓ cultured mammary basal tumor cells lacking α3β1 were unable to grow in soft agar and were much less able to proliferate in 3D Matrigel cultures or to develop tumors in orthotopic grafts
- ✓ MAPK and JNK, similar to Rac1, PAK1 FAK, are intermediates of α3β1 integrin signaling required for tumor development
- ✓ α3β1 integrin was found to be required for the tumor cell invasion and the stimulation of angiogenic endothelial cell function. These data suggest that α3β1 integrin can influence various aspects of tumorigenesis by controlling different cancer cell functions
- ✓ expression of α3β1 integrin appeared to be upregulated in mammary lesions of K5DNbcat mice, this integrin could be targeted in anticancer therapy

Thanks for
your attention.